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Strategies to Address Infection Prevention and Treatment in the Reduced Inflammatory Milieu of Irrigated Open Wound

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14. ABSTRACT

Open fractures require irrigation and debridement to prevent potential infection. Irrigation and debridement, however, removes the body's first healing response, such as fracture hematoma. We implemented an irrigated radius defect model intended to represent the open fracture setting with bone loss. We evaluated feasibility to investigate the role of platelet rich plasma (PRP) to restore healing response. We completed the hypothesis development protocol evaluating the separate and combined roles of irrigation and PRP augmentation in a rabbit radius defect model. Irrigation was with commercially available pulse lavage. PRP was manually prepared with centrifugation and separation. Platelet concentration was compared to whole blood platelet concentration. PRP was combined with thrombin prior to insertion in the defect. Healing was assessed radiographically, histologically and with microCT. Intended power was not achieved due to animal loss (ulnar fracture and due to unrelated intestinal causes). Platelet concentration exhibited variability across all rabbits, from 2x to 8x. Histologic scoring and radiographic evaluation did not confirm that irrigation reduced healing potential, at 3 and 6 weeks, but sample number was compromised and results were not definitive. Future work will evaluate one time point, fibrinogen will be used to avoid quick activation of thrombin, and the ulnar defect will be considered to avoid inadvertent forearm fracture.

15. SUBJECT TERMS
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Table of Contents

Introduction:

Open fractures have an increased incidence of infection due to environmental contamination. Irrigation and debridement has been proven to reduce infection; however, these procedures also remove beneficial mediators that initiate and drive the healing process in orthopaedic injuries. As a result, open fractures have a higher probability of delayed healing or non-union fractures.

There are several possible methods to address the delayed healing following irrigation and debridement. A method that may re-introduce some of the factors lost during I&D is autologous platelet rich plasma (PRP). PRP is made when whole blood is processed, by concentrating platelets and their growth factors. PRP is approved by FDA for clinical use in patients and can be prepared by simple centrifugation. Adding PRP to the defect after irrigation of the wound may be a way to improve healing.

This study is designed as an initial step of applying PRP to irrigated and non-irrigated bone defects to determine if PRP has the potential to improve the healing of open fractures. The immediate goal was to gather preliminary data to generate a hypothesis for future experimental protocols.

Body:

It has been shown that growth factors such as transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) induce chemotaxsis and mitogenisis of inflammatory cells, fibroblasts, and osteoprogenitor cells. These growth factors and others are packaged within alpha granules of platelets and are critical to the initiation and maintenance of the body's injury response mechanism.

When tissue is damaged, platelets are exposed to von Willebrand factor, collagen, tissue factor, and thrombin (formed in the clotting process); become activated; and release their contents to include platelet alpha granules into the defect site. When these alpha granules are released (platelet degranulation) the growth factors contained induce both a paracrine and endocrine signaling effect on key cellular components that fight potential infection; clear damaged, necrotic, and foreign material; and begin cellular repair.

Current strategies in open fracture management include irrigation of the site to remove potential foreign bodies, contaminants, and infectious agents. While this strategy reduces the potential for infection and granuloma formation, it also has the potential to remove favorable growth factors released from activated platelets and can hinder the overall healing of the defect site.

From our understanding of the importance and function of growth factors contained in platelet alpha granules, it can be postulated that an infusion of

additional growth factors into a boney defect would catalyze the healing process by amplifying chemotactic and mitogenic signaling needed for wound repair, especially in open fractures where key growth factors can be removed during treatment. One method that is already FDA approved is the application of platelet-rich plasma (PRP). PRP is an autologous whole blood derivative composed of concentrated platelets, activated externally by thrombin, and infused or injected into soft tissue, tendon, ligament, and boney defects. PRP is activated by thrombin to ensure platelet degranulation and subsequent growth factor release. Current studies have shown that PRP can be efficacious in some soft tissue injury; however, the potential of PRP application in orthopaedic trauma is not well understood.

This protocol was specifically designed to address the application of PRP in a defect model representing an open bony defect; however, our research spurred an equally important tangential study using our collected data. Here we documented and analyzed the initial variability of platelets in rabbit whole blood and PRP preparations to help define the range of variability in PRP prepared in a controlled setting.

Initially, we looked at the initial whole blood platelet concentration in the female New Zealand White Rabbit model. We found in our study of 52 rabbits (56 initially and 4 were omitted as statistical outliers) the initial whole blood platelet concentration ranged from 121 to 452 x 10^3/μL. Previous studies have reported initial platelet concentrations of the New Zealand white rabbit ranging from 250 – 750 x 10^3/μL[ref]. It is critical to document and analyze this variance, because even though PRP is an FDA approved treatment modality, the definition of PRP is relatively ambiguous.

The currently accepted definition of PRP is simply “plasma enriched with platelets[ref].” There are no defined parameters suggesting an enrichment goal (platelet concentration above baseline or absolute value of platelets). A consequence of the absence of standardization is that inconstant results in PRP studies could be a result of PRP variance and/or the use of sub-optimal growth factor concentration in bone and soft-tissue defects. For instance in our study, PRP preparations ranged from 2.89 to 8.80 x baseline values. We attempted to standardize platelet concentration to 4 x baseline values; however, this proved to be difficult as agitation of PRP induced platelet degranulation. We ultimately determined this variability in platelet concentration was a confounding factor in the data, and would need to be analyzed as such in subsequent studies.

Our PRP variability arm of this study documents the initial PRP concentrations of our animal model. We then examined our data for the following:

1) Correlation between PRP platelet concentration and arterial whole blood platelet concentration

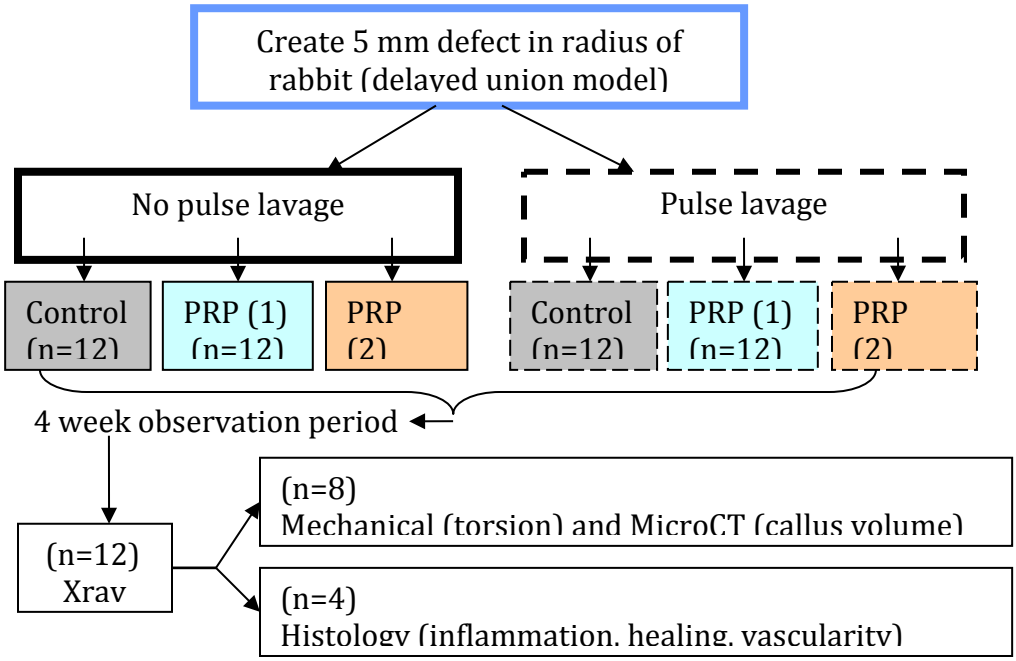
2) Correlation between arterial whole blood platelet concentration and rabbit weight
3) Correlation between arterial whole blood platelet concentration and rabbit age

This is intended to provide the initial step in understanding platelet and PRP variability and ultimately work toward defining successful PRP preparations.

Appendix 1 - Platelet variability results

Supporting Data 1 - Subject initial whole blood and PRP platelet concentrations

The primary intent of our study was hypothesis development, which will be used to drive future protocol to evaluate PRP in irrigated and debrided orthopaedic injuries. Our protocol is described in the chart below:



This study met our research goal of driving the development follow-on studies to study methods to treat open, irrigated fractures, here with PRP.

Our preliminary data (insufficient power for definitive conclusions) suggests that, pulsitile lavage in the defect produced favorable results at 6 week follow up. In addition, the application of PRP after pulsatile lavage was shown to have the highest overall mean histological score at 6 weeks.

If we account for statistical outliers, we found that there was a significant difference between the 6 week no irrigation with PRP (histological score of 12.00) vs the 6 week irrigation without PRP (histological score of 5.33). This suggests

that PRP is best utilized after defect irrigation, possible due to the removal of growth factors within the defect site.

Is it important to emphasize that this study lacked overall statistical power to make any definitive conclusions. The intent was to drive future models and determine if PRP could potentially catalyze the healing process of bone fractures.

One reason our study lacked significant statistical power was due to rabbit loss despite our best veterinary efforts. Rabbits were purchased through two different vendors to meet our research requirements. The two vendors (Charles River Canada and Bakkom Rabbitry) provided New Zealand White (NZW) rabbits, but the Bakkom rabbits are considered conventional and the Charles River rabbits are bred free of Clostridium spiroforme and Pasteurellosis infections. A member of our institutional IACUC is a veterinarian with substantial experience with rabbits as experimental models, suggested that it was a possibility that these rabbits may have had Clostridium infections or that their age resulted in their death. Further consultation at the University of Minnesota Small Animal Department (College of Veterinary Medicine) emphasized that the impacted fur in stomachs could occur due to room temperature.

As our facility has not experienced rabbit deaths in the past, we do not anticipate that our veterinary staff would have recurrent difficulties in maintaining rabbits during follow up postoperatively. We would plan to have strict control of room temperature and would choose Charles River rats preferentially.

Supporting Data 2 - Histological grading outline

Supporting Data 3 – Histological grading results

Supporting Data 4 - Statistical analysis of results

Key Research Accomplishments:

- The irrigated rabbit ulna defect model was implemented for the study of PRP.

- PRP preparation was uneventful, but there was substantial variation in the number and fold-increase in platelets in the PRP.

- Thrombin activation of PRP has recently been challenged as a method to provide gelling of the PRP, with the criticism that it may cause too rapid expulsion of the contents of the platelets. We intend to evaluate the use of fibrinogen in future studies.

- Radiological, histologic and microCT outcome measures were successfully obtained.

- Forearm fracture following radial defect preparation was observed. In consultation with other researchers using both radius defect and ulnar defect model, we will consider use of ulnar defect in the future (leaving the stronger radius as the remaining forearm stabilizer).

- Intestinal blockage caused fatalities in rabbits. With consultation of IACUC and veterinarian researchers, room temperature will be closely monitored in future studies.

- We evaluated two time points, 3 and 6 weeks. There were not substantial differences in the two observation periods, and in future studies we intend to evaluate one time period, likely 4 weeks.

Reportable Outcomes:

- Hongnaparak, T; Johnson, W.S.; Tsukayama, D.T.; Bechtold, J.E. *Distribution of Platelet Concentration in Whole Blood and Platelet-Rich Plasma*
- 2012 Military Health System Research Symposium (MHSRS) poster (Florida)
- 2012 Orthopaedic Research Society annual meeting abstract presentation (San Francisco, CA)
- 2011 Best of Hennepin County Medical Center poster session (Minneapolis, MN)

- We are currently finalizing a scholarly article on our platelet variability study and intend to formally publish our results to the scientific community.

- Paid Personnel: Dean Tsukayama, MD, Joan Bechtold, PhD, Barbara Wicklund, BS, Toni Meglitsch

- Unpaid Personnel: Theerawit Hongnaparak M.D. – Dr. Hongnaparak is an orthopaedic surgeon from Thailand that completed the Hennepin County Medical Center Orthopaedic Surgery Research fellowship while on this project; Capt William S. Johnson – Universtity of Minnesota medical student and member of the Minnesota Air National Guard medical corps was a member of this project and received credit toward his M.D. degree (anticipated, 2014) while on his orthopaedic surgery research clerkship.

Conclusion:

We have completed the hypothesis development protocol evaluating the separate and combined roles of irrigation and PRP augmentation in a rabbit radius defect model. There was loss of power due to animal loss from post-op forearm fracture and unrelated intestinal blockage. Despite the lack of

significance in findings for separate effects of irrigation and PRP, there was a positive effect of PRP when used in irrigated setting. Future work investigating hypotheses of reduced healing with irrigation and restoration of healing with PRP will evaluate one time point, fibrinogen will be used to avoid quick activation of thrombin, and the ulnar defect will be considered to avoid inadvertent forearm fracture.

References:
See Appendix 1

Appendix:
See Below

Appendix 1

Distribution of Platelet Concentration in Whole Blood and Platelet-Rich Plasma

[1]Hongnaparak, T; +[2]Johnson, W S; [1,2,3]Bechtold, J E; [2,3]Tsukayama, D T
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INTRODUCTION:

While the healing effects have not been consistent in multiple models and studies, Autologous Platelet-Rich Plasma (PRP) has been shown in some studies to improve soft tissue and bone healing.[1-7] It is presumed that any positive effect is achieved by delivering a concentrated amount of growth factors such as transforming growth factor-ß1 (TGF- ß1); platelet-derived growth factor (PDGF) – AA, AB, BB; and vascular endothelial growth factor (VEGF), contained in platelet α-granules. These growth factors, among others induce chemotaxis to and mitogenesis of macrophages, fibroblasts, and osteoprogenitor cells within the wound and fracture.[1,6,8]

In this study our aim was to compare platelet concentration variability in whole blood (WB) and manually prepared PRP in the female New Zealand White rabbit model. In addition, we set out to examine the following:

1. Correlation between PRP platelet concentration and arterial whole blood platelet concentration
2. Correlation between arterial whole blood platelet concentration and rabbit weight
3. Correlation between arterial whole blood platelet concentration and rabbit age

METHODS:

This study protocol was approved by our institutional IACUC. 56, 3-5 kg female New Zealand White Rabbits ages 6 – 33 months were identified for whole blood platelet analysis and potential PRP treatment.[8] Immediately following general anesthesia, a 17-gauge needle was inserted into the central auricular artery and 10 ml autologous arterial blood was drawn into a sterile citrate tube. PRP was manually prepared using two centrifugations (300g for 10 minutes to separate out red blood cells and 5,000g for 5 minutes to separate out platelet poor plasma).[7-9] Whole blood and PRP platelet concentration was quantified by standard veterinary laboratory analysis.

Statistical analysis was performed using SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC; USA, 2008). Four samples were excluded from the study (two due to the formation of clots during PRP preparation and two data points were identified as statistical outliers).

RESULTS:

Table 1: Mean Values, Ranges, and Enrichment of Platelets (PLT)[§]

	PLT concentration (x 10^3/μL)*	PLT range (x 10^3/μL)	Enrichment range
Arterial WB	247 ± 83	121 - 452	-
PRP	1188 ± 421	497 - 2409	2.89 - 8.80

[§]n = 52, *mean ± SD

Table 1 represents our observed mean values ± standard deviation and ranges of platelet concentration in rabbit arterial whole blood and PRP, and enrichment concentration range of PRP (PRP/WB).

There was a positive correlation between the arterial whole blood platelet concentration and prepared PRP platelet concentration (R=0.769, P < 0.05) shown in Figure 1.

There was a weak positive correlation between the age of the rabbit and arterial whole blood platelet concentration (R=0.397, P < 0.05) shown in Figure 2.

There was no statistical correlation between arterial whole blood platelet concentration and the weight of the rabbit at the time of blood draw (P < 0.05).

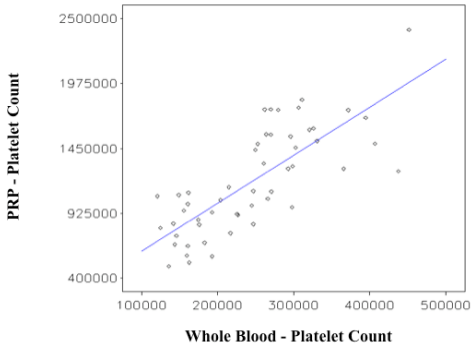

Fig. 1. Positive correlation between PRP platelet concentration and initial whole blood platelet concentration (R=0.769, P=0.05)

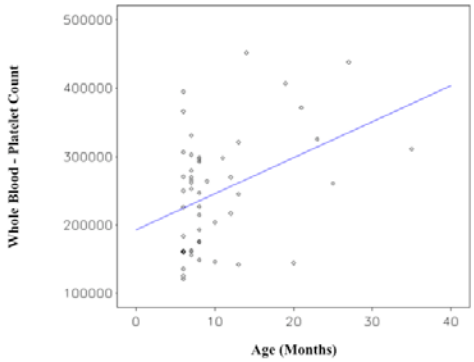

Fig. 2. Positive correlation between whole blood platelet concentration and rabbit age (R=0.397, P=0.05)

DISCUSSION:

We observed an arterial whole blood platelet concentration range of 121 – 452 x 10^3 platelets/μL. Current literature suggests New Zealand White rabbit venous whole blood platelet concentration ranges from 250 - 750 x 10^3 platelets/μL and human whole blood platelet concentration ranges from 150-450 x 10^3 platelets/μL.[7,10-14]

As expected, there was a strong positive correlation between PRP platelet concentration and arterial whole blood platelet concentration (R=0.769, P<0.05) as shown in Figure 1. Given the variation in platelet number in whole blood, for the same platelet concentration percentage, the total number of platelets in the PRP is expected to vary.

A weak positive correlation between whole blood platelet concentration and rabbit age was observed as shown in Figure 2. Future studies should investigate this observation in greater detail.[12,13]

It is outside the scope of this study to examine arterial whole blood platelet concentration drawn from the central auricular artery compared to venous whole blood platelet concentration drawn from the marginal ear vein, though data suggests that there may be a significant difference in platelet concentration between the two.[2,4,6-8,10] Future models and studies should investigate this issue.

SIGNIFICANCE:

Understanding platelet variability in whole blood and PRP will allow clinicians and researchers to develop standardized PRP protocol and better understand the application of PRP in bone healing.

ACKNOWLEDGEMENTS:

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Appendix 1

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Supporting Data 1 - Subject Initial Whole Blood and PRP Platelet Concentrations

No	Name	Whole Blood	PRP	PRP/WB	Note
1	B-02	247,000	837,000	3.39	
2	B-03	298,000	976,000	3.28	
3	B-09	245,000	989,000	4.04	
4	B-10	438,000	1,268,000	2.89	
5	B-11	450,000	726,000	1.61	Statistical outlier
6	B-12	193,000	577,000	2.99	
7	B-13	404,000	746,000	1.85	Statistical outlier
8	B-15	266,000	1,043,000	3.92	
9	B-16	226,000	918,000	4.06	
10	B-21	161,000	662,000	4.11	
11	B-22	183,000	687,000	3.75	
12	B-23	366,000	1,288,000	3.52	
13	B-24	136,000	497,000	3.65	
14	B-25	161,000	1,000,000	6.21	
15	B-26	163,000	528,000	3.24	
16	B-27	146,000	743,000	5.09	
17	B-28	160,000	583,000	3.64	
18	B-29	372,000	1,760,000	4.73	
19	B-30	227,000	913,000	4.02	
20	B-31	331,000	1,510,000	4.56	
22	B-33	156,000	948,000	6.08	
23	B-34	270,000	1,562,000	5.79	
24	B-35	142,000	842,000	5.93	
25	B-36	321,000	1,601,000	4.99	
26	B-37	326,000	1,613,000	4.95	
27	B-38	217,000	763,000	3.52	
28	B-39	204,000	1,032,000	5.06	
30	B-41	215,000	1,137,000	5.29	
31	B-42	149,000	1,073,000	7.20	
32	B-43	247,000	1,106,000	4.48	
33	B-44	261,000	1,330,000	5.10	
34	B-45	299,000	1,309,000	4.38	
35	B-46	175,000	874,000	4.99	
36	B-47	176,000	832,000	4.73	
37	B-48	193,000	935,000	4.84	
38	B-49	144,000	672,000	4.67	
39	B-50	407,000	1,490,000	3.66	
40	B-51	293,000	1,287,000	4.39	
41	B-52	125,000	808,000	6.46	
42	B-54	264,000	1,566,000	5.93	
43	B-55	303,000	1,459,000	4.82	
44	B-56	296,000	1,548,000	5.23	
45	B-57	452,000	2,409,000	5.33	
46	B-64	121,000	1,065,000	8.80	
47	B-65	250,000	1,440,000	5.76	
48	B-84	311,000	1,846,000	5.94	
49	B-66	271,000	1,102,000	4.07	
50	B-67	395,000	1,700,000	4.30	
51	B-68	307,000	1,782,000	5.80	
52	B-69	162,000	1,091,000	6.73	
53	B-76	262,000	1,766,000	6.74	
54	B-77	270,000	1,768,000	6.55	
55	B-78	280,000	1,764,000	6.30	
56	B-79	253,000	1,488,000	5.88	

Supporting Data 2 - Histological Grading Outline

Healing within confines of the defect (within rectangle defined by each of 4 outer cortices at the margins of the defect)	0- no new bone/cartilage 1- up to 25% new bone/cartilage 2- between 25-49% new bone/ cartilage 3- 50% and greater to 74% new bone/cartilage 4- 75% new bone/cartilage and greater
Tissue Type within defect	0- 75-100% fibrous connective tissue 1- 50-74% fibrous connective tissue 2- 25-49% fibrous connective tissue 3- less than 24% connective tissue 4- No fibrous connective tissue present
Boney bridge connecting the two cortices across the defect	0- none 1- up to 25% 2- 25-50% 3- 50-75% 4- Complete
Periosteal bridging across defect	0- none on either side of defect 1- partial one side 2- partial both sides 3- complete on one side 4- complete on both sides

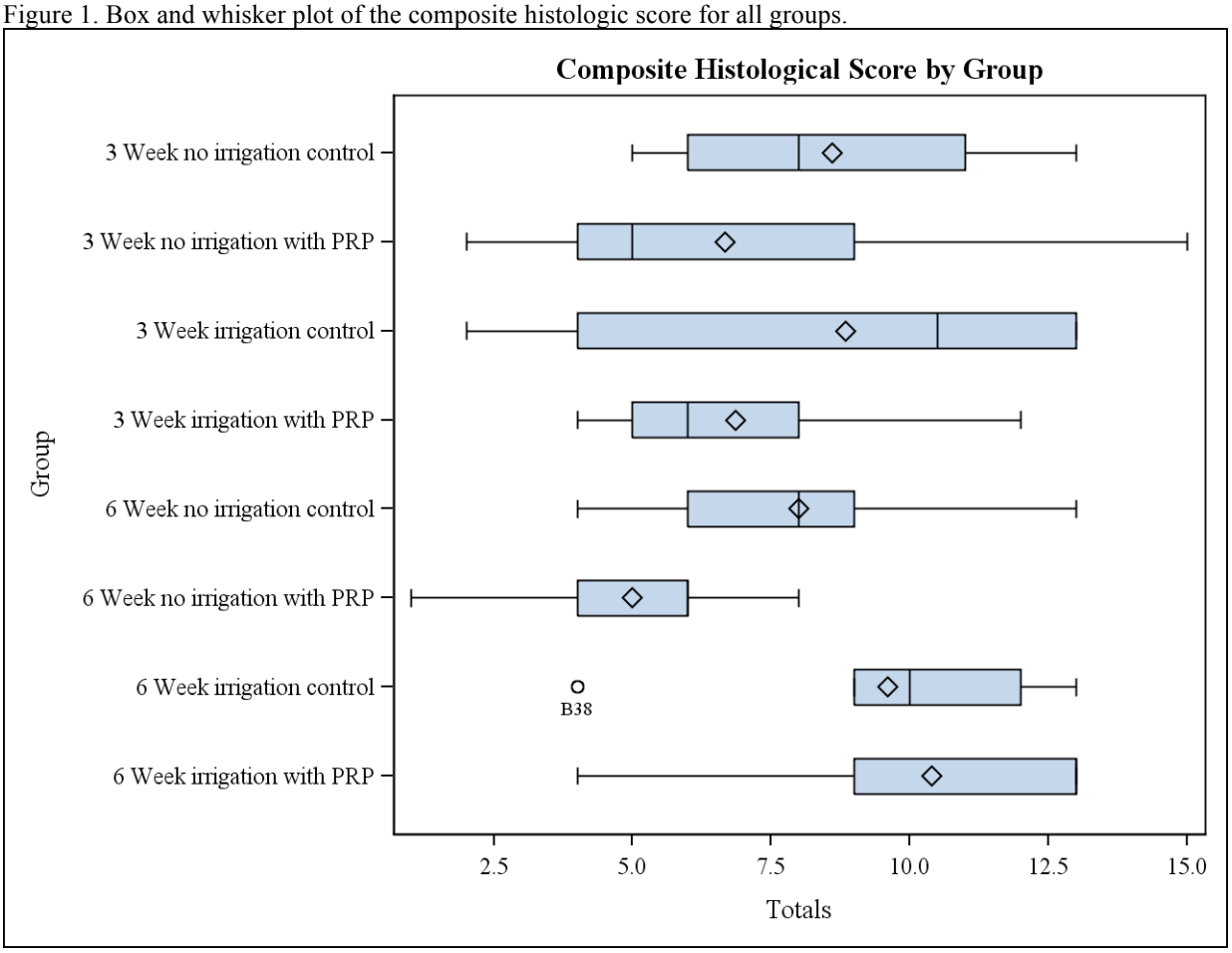
Supporting Data 3 - Histological Grading Results

Project Rabbit Number and Group	Healing	Fibrous tissue	Bridging in defect	periosteal bridiging	Totals
	Control 3 week no irrigation	3 week irrigation control	6 week no irrigation control	6 week irrigation control	
	PRP no irritagion 3 weeks	3 week irrigation PRP	6 week no irrigation PRP	6 week irrigation PRP	
B19	4.0	3.0	3.0	3.0	13.0
B20	3.0	2.0	0.0	1.0	6.0
B24	4.0	3.0	3.0	1.0	11.0
B28	4.0	3.0	0.0	1.0	8.0
B11	1.0	0.0	1.0	3.0	5.0
Control 3 week no irrigation average:	3.2	2.2	1.4	1.8	8.6
B15	3.0	2.0	1.0	3.0	9.0
B22	1.0	0.0	0.0	1.0	2.0
B29	3.0	2.0	1.0	0.0	6.0
B9	2.0	1.0	0.0	1.0	4.0
B02	2.0	1.0	0.0	1.0	4.0
B03	4.0	4.0	4.0	3.0	15.0
PRP no irritagion 3 weeks average	2.5	1.7	1.0	1.5	6.7
B13	3.0	2.0	0.0	3.0	8.0
B17	4.0	3.0	3.0	3.0	13.0
B18	4.0	3.0	3.0	3.0	13.0
B21	1.0	0.0	0.0	1.0	2.0
B30	4.0	3.0	3.0	3.0	13.0
B12	2.0	1.0	0.0	1.0	4.0
3 week irrigation control average	3.0	2.0	1.5	2.3	8.8
B16	4.0	3.0	3.0	2.0	12.0
B23	2.0	1.0	0.0	1.0	4.0
B25	1.0	0.0	1.0	3.0	5.0
B27	3.0	2.0	0.0	2.0	7.0
B31	3.0	2.0	0.0	1.0	6.0
B10	2.0	1.0	2.0	3.0	8.0
B44	3.0	2.0	0.0	1.0	6.0
3 week irrigation PRP average	2.6	1.6	0.9	1.9	6.9
B35	2.0	1.0	0.0	1.0	4.0
B40	4.0	3.0	0.0	2.0	9.0
B49	3.0	2.0	0.0	1.0	6.0
B52	3.0	2.0	0.0	3.0	8.0
B55	4.0	3.0	3.0	3.0	13.0
6 week no irrigation control average	3.2	2.2	0.6	2.0	8.0
B41	3.0	2.0	0.0	1.0	6.0
B43	1.0	0.0	0.0	0.0	1.0
B46	3.0	2.0	0.0	1.0	6.0
B47	2.0	1.0	0.0	1.0	4.0
B33	3.0	2.0	0.0	3.0	8.0
6 week no irrigation PRP average	2.4	1.4	0.0	1.2	5.0
B38	2.0	1.0	0.0	1.0	4.0
B50	4.0	3.0	1.0	2.0	10.0
B60	4.0	3.0	3.0	3.0	13.0
B70	3.0	2.0	1.0	3.0	9.0
B71	4.0	3.0	2.0	3.0	12.0
6 week irrigation control average	3.4	2.4	1.4	2.4	9.6
B34	4.0	3.0	3.0	3.0	13.0
B37	2.0	1.0	0.0	1.0	4.0
B45	4.0	3.0	3.0	3.0	13.0
B48	4.0	3.0	3.0	3.0	13.0
B54	4.0	3.0	1.0	1.0	9.0
6 week irrigation PRP average	3.6	2.6	2.0	2.2	10.4

Supporting Data 4 - Statistical Analysis of Results

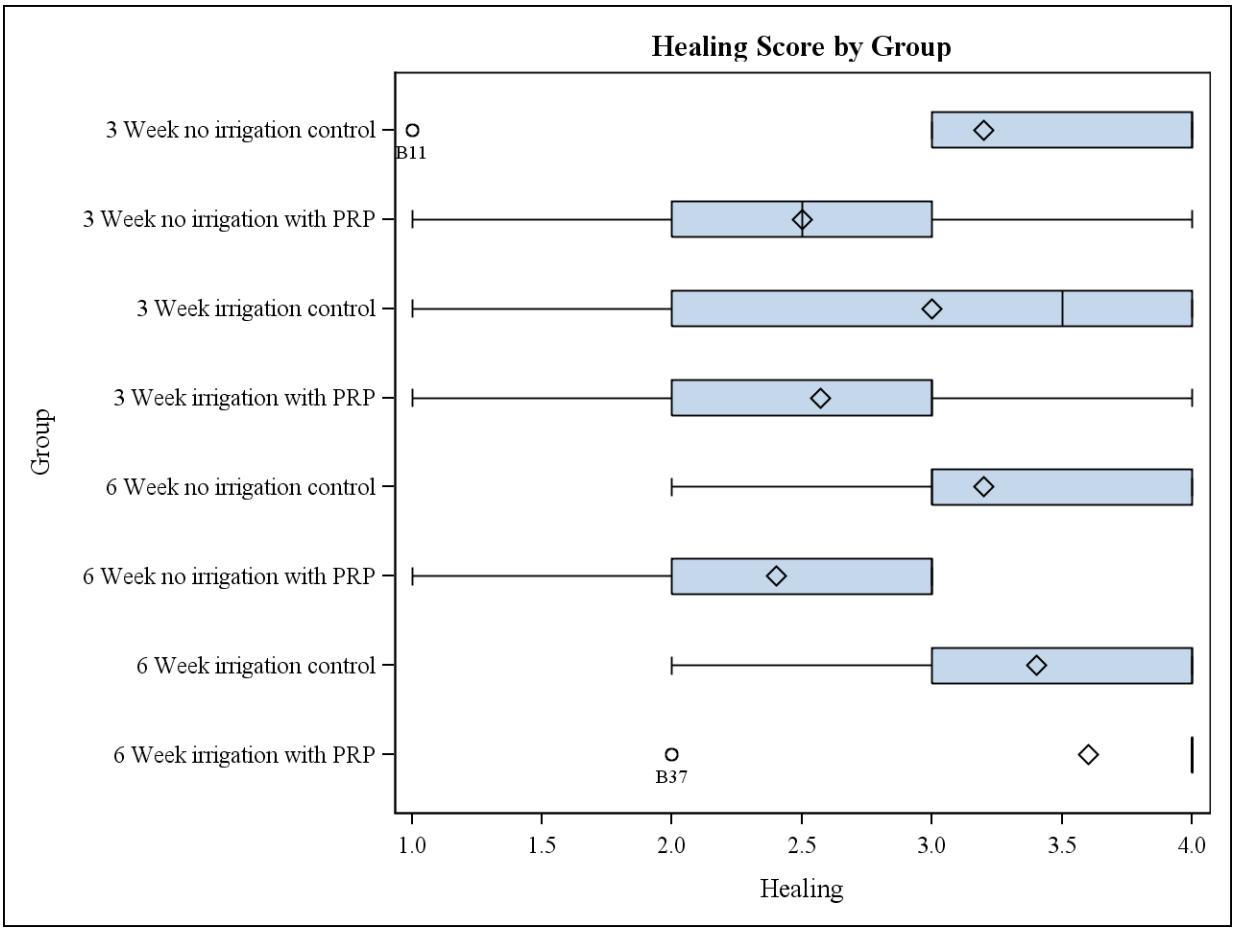
Section 1. Identifying groups outliers for all histological scores.

Figure 1. Box and whisker plot of the composite histologic score for all groups.



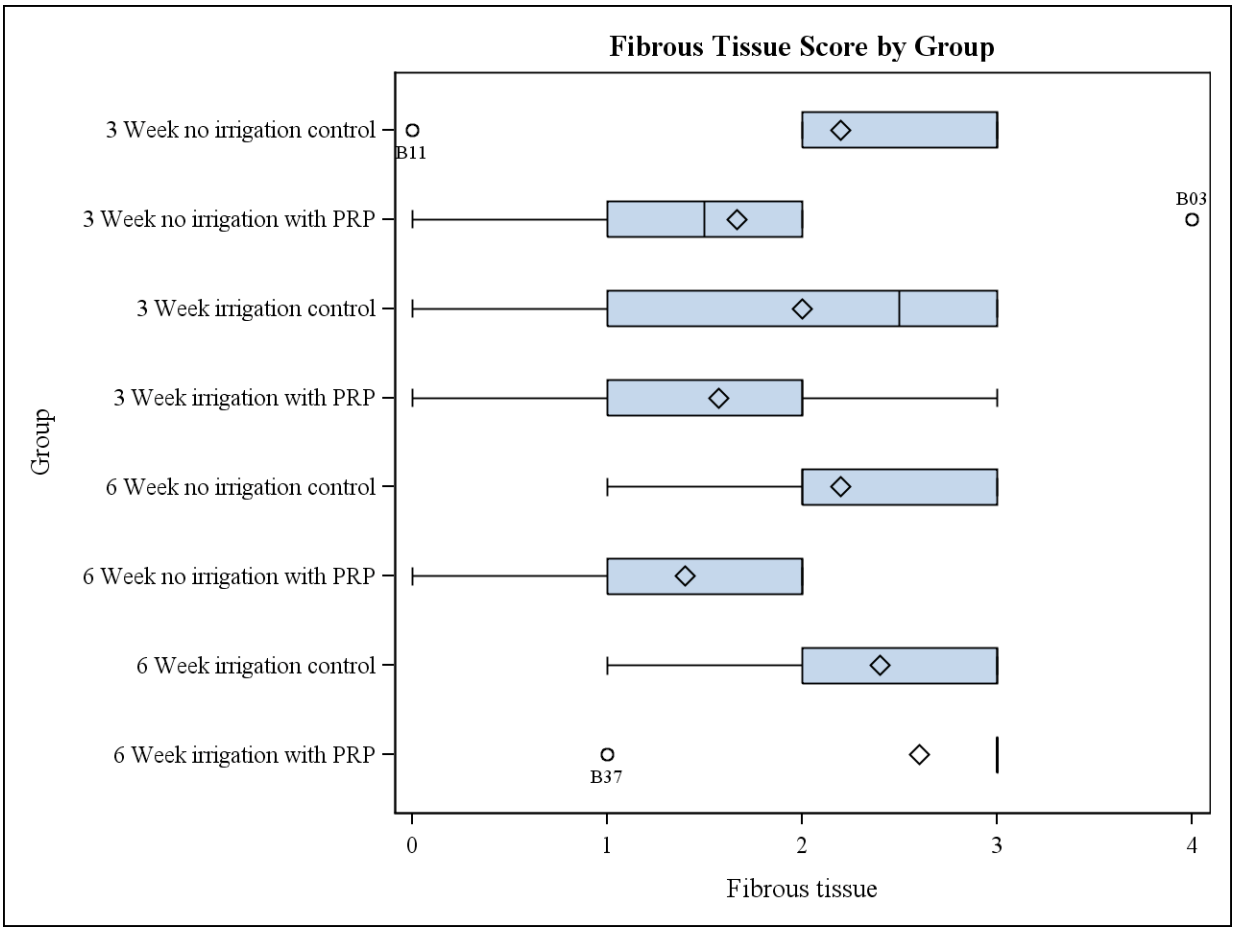
B38 was an outlying observation within its group.

Figure 2. Box and Whisker plot of Healing Score for each group



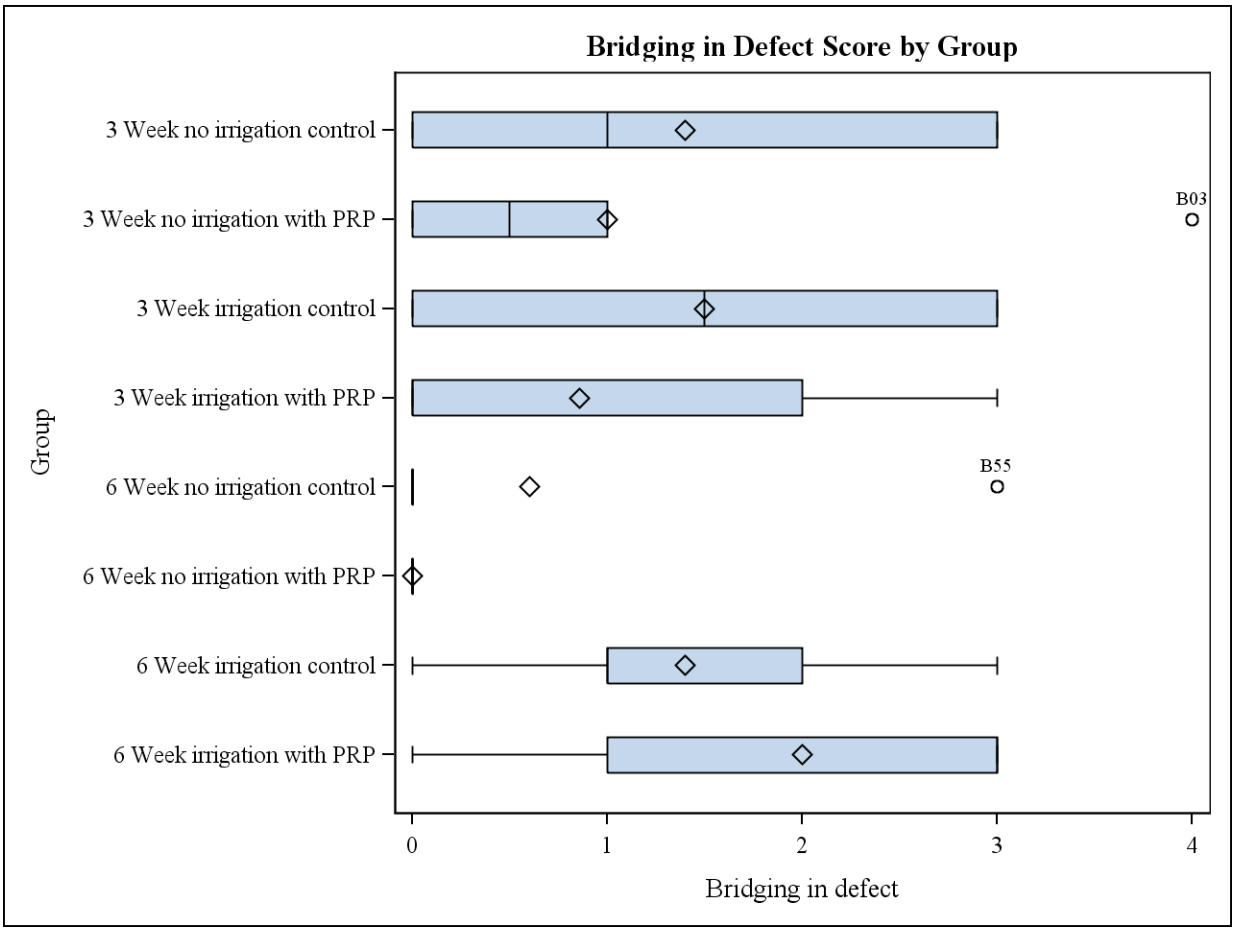
There are two outlying observations: B11 and B37.

Figure 3. Box and Whisker plot of Fibrous Tissue score for each group



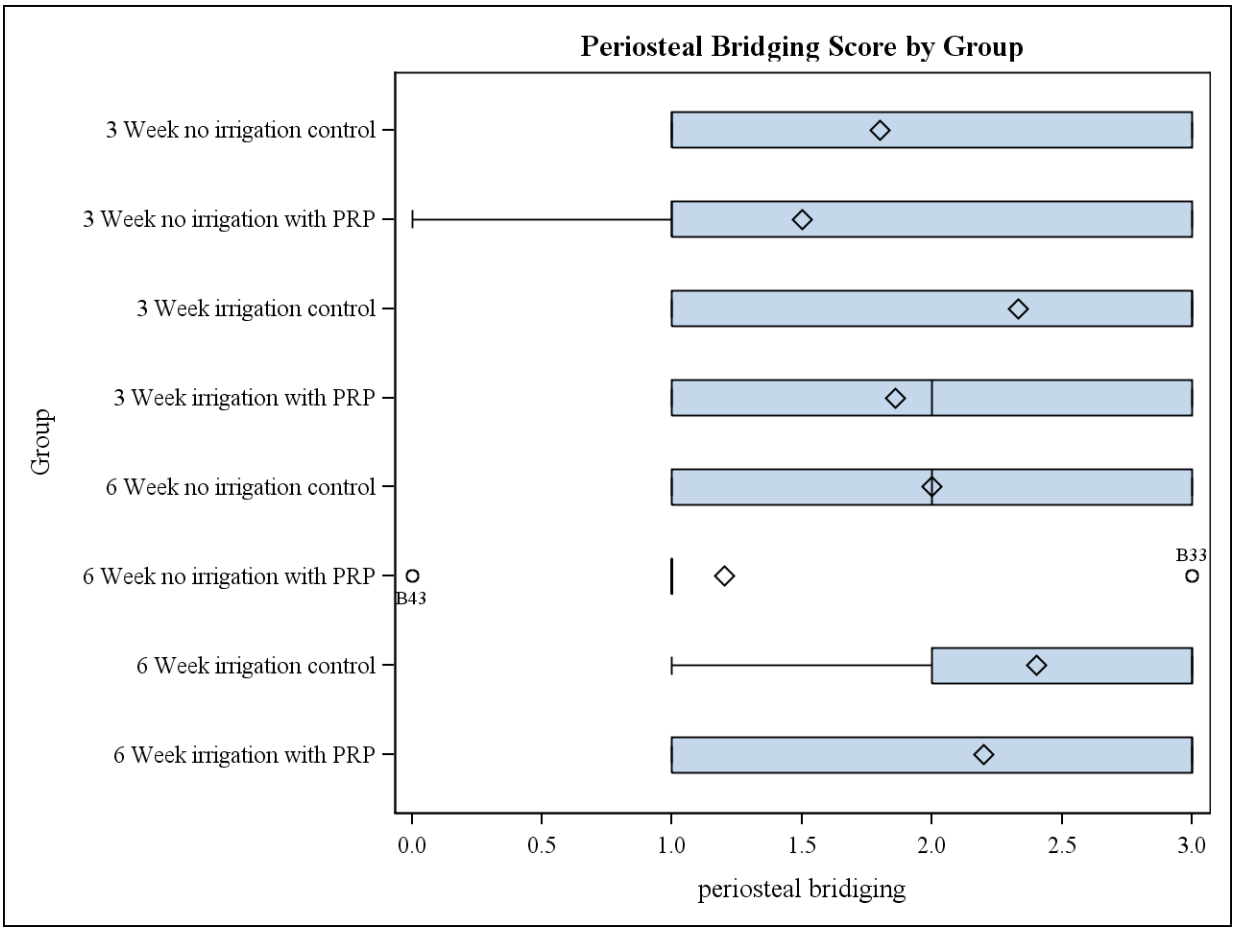
B11, B03, and B37 were all group outliers.

Table 4. Box and Whisker plot of bridging in defect score for all groups



B55 and B03 were both group outliers.

Figure 5. Box and Whisker plot of Periosteal Bridging by group



B44 and B33 were both group outliers for the 6 Week no irrigation with PRP group.

Summary: For total score, only one group outlier was identified: B38. For all the four components of the histological score, there were several group outliers: B03, B11, B33, B37, B38, B43, and B55.

Section 2: Analysis Results

Before summarizing the analysis results, I want to mention a few things:

- It typically is not good practice to throw out data points even though they might be outliers, especially when experiments have small sample sizes (such as this one). However, I do realize that it is often desirable to explore results without outlying data points.
- In cases where it is desirable to exclude certain data points, its best to do a comparison of the results both with and without outliers: if the results are the same or similar, that can help assure us that excluding the data points did not influence the results.
- Since you are most interested in detecting differences between the groups in regard to the composite score, I felt it was more appropriate to identify outliers in the composite score rather than in the four components scores. However, I did an analysis both ways: one that excluded outliers for the total score, and one that excluded outliers for each of the component scores.

As a results, three models were run:

- One that used data from all rabbits
- One that excluded outliers for the total score (all rabbits but B38)
- Lastly, one that excluded outliers for each of the component scores

All models were run in SAS Version 9.2. A one-way ANOVA model was used to detect differences in the total score between all groups. Type I error was controlled by adjusting p-values for multiple comparisons using simulation-based methods[1].

Table 1. Group Total Means Scores

	All Data		**Composite Score Outliers Excluded** (B38 only)		**Histological Component Outliers Excluded (**B03, B11, B33, B37, B38, B43, and B55)	
Group	**Average**	**Standard Error**	**Average**	**Standard Error**	**Average**	**Standard Error**
3 Week irrigation control	8.83	1.53	8.83	1.49	8.83	1.22
3 Week irrigation with PRP	6.86	1.42	6.86	1.38	6.86	1.13
3 Week no irrigation control	8.6	1.67	8.6	1.63	9.50	1.50
3 Week no irrigation with PRP	6.67	1.53	6.67	1.49	5.00	1.34
6 Week irrigation control	9.60	1.67	11.00	1.83	11.00	1.50
6 Week irrigation with PRP	10.40	1.67	10.40	1.63	12.00	1.50
6 Week no irrigation control	8.0	1.67	8.0	1.63	6.75	1.50
6 Week no irrigation with PRP	5.0	1.67	5.0	1.63	5.33	1.73

- The average total score varied for each group under the three scenarios: the 6 week no irrigation with PRP group had the lowest score in both “All data” and “Composite Score Outliers Excluded” scenarios, but the 3 week no irrigation with PRP group had the lowest score in the “Histological Component Outliers Excluded” scenario. The 6 week irrigation with PRP group had the highest total score in the “All data” and “Histological Component Outliers Excluded”, but the 6 week irrigation control group had the highest score in the “Composite Score Outliers Excluded” scenario.